Freshwater Mussel Survey Results
West Fork Busseron Creek Mitigation Area
Farmersburg, Indiana

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Executive Summary

A freshwater mussel survey was conducted by ENVIRON International Corporation (ENVIRON) within the West Fork Busseron Creek Mitigation Area (WFBCMA) near Farmersburg, Sullivan County, Indiana. The WFBCMA is a post-mining reclamation action completed in 2006 by Peabody Midwest Mining, LLC, in the West Fork Busseron Creek. Pre-mining bioassessment results indicated no mussels to be present in this portion of West Fork Busseron Creek. However, during post-reclamation bioassessment monitoring of fish and benthic macroinvertebrate, specimens of freshwater mussels were observed in the WFBCMA. This report presents the results of a mussel survey conducted on August 5, 2011 to characterize and evaluate freshwater mussels in the WFBCMA.

The mussel survey in the WFBCMA used standard mussel survey methods based on systematic sampling from randomly selected survey plots associated with a stratified distribution of cross-stream transects in a stream study reach. The WFBCMA survey incorporated two study stream reaches a minimum of 150 feet in length located near the upstream and downstream portions of the mitigation area. Stream sediment from within a 0.25 M^2 survey plot was removed to a depth of approximately 4-5 inches and sieved through a ¼ inch screen. All mussels encountered were identified to species, measured (total length), and counted prior to replacement in the stream. Key findings of the mussel survey in the WFBCMA include the following:

1. *Ligumia subrostrata* (Pondmussel) and *Utterbackia imbecillis* (Paper Pondmussel) are present in the upper portion of the WFBCMA.
2. A total of 39 stream survey plots were evaluated for mussels. Extrapolated total mussel density was 3.4 mussels/M^2 with *Ligumia subrostrata* density being double that of *Utterbackia imbecillis*.
3. Analysis of annular shell growth rings and comparison to length measurements suggest that mussels have been colonizing the upper portion of the WFBCMA for at least two to three years. Maximum valve length was 85.3 mm for *U. imbecillis* and 33.7 mm for *L. subrostrata*.
4. The downstream study reach was positioned below a recently constructed and active beaver dam. No mussels were encountered in any of 10 stream survey plots from below the beaver dam. Construction of the beaver dam and recent deposition in the streambed from an episodic event, each occurring after the 2010 bioassessment, may have contributed to the lack of mussels in this portion of the WFBCMA.
5. Mean field measured water quality parameters or pH (7.5 su), dissolved oxygen (8.5 mg/L), temperature (32 °C), and specific conductivity (275 mS/cm) indicated warm water conditions consistent with the geographical area. However, the lowest dissolved oxygen concentration and a lowering of water temperature appeared to a consequence of the beaver dam.

Given the complex life cycle and requirements for freshwater mussels to occur in streams, the presence of multiple Unionid mussel species in the WFBCMA is further evidence that biological function of the stream has been attained.

Introduction

ENVIRON International Corporation (ENVIRON) conducted a freshwater mussel survey within the West Fork Busseron Creek Mitigation Area (WFBMA), Farmersburg, Sullivan County, Indiana. Peabody Midwest Mining, LLC (Peabody) established the WFBMA as reclamation following of mining activities at the Farmersburg Mine. Bioassessment of the West Fork Busseron Creek prior to mining disturbance included a formal mussel survey, from which no mussel specimens were reported (Three Rivers 2003 *Biological Inventory and Substrate Classifications in West Fork Busseron Creek, Sullivan County Indiana*). The WFBCM area is approximately 7,825 feet in length and stream reconstruction plans incorporated current aspects and understanding of hydrology and stream morphology to enhance the ecological benefits of the stream specific to the gradient and geographical area.

Four years following reclamation, ENVIRON conducted a benthic macroinvertebrate and fish bioassessment in the WFBCMA (June 29-July 1, 2010). The bioassessment provided biological information as a temporal benchmark for demonstrating community composition and functional aspects of the stream which were equivalent with pre-mining conditions. During this bioassessment, ENVIRON incidentally observed the presence of Unionid freshwater mussels within in the study area.

This 2011 mussel survey was conducted to verify and quantify the presence of freshwater mussels in the WFBMA following Peabody Energy's reclamation actions. The distribution and abundance of freshwater mussels has been a recent focus of environmental and ecological concern of state, federal, and special interest groups. Documentation of a freshwater mussel population in the WFBCMA is a strong ecological statement that further demonstrates successful mitigation resulting in good water quality, and restoration of stream function.

Methods

Water Quality

Water quality measurements were recorded in situ using a portable Horiba water quality multi-probe meter. Parameters, including pH (s.u), dissolved oxygen (mg/L), conductivity (µmhos/cm), and temperature (°C), were taken intermittently during the day to account for any diurnal fluctuation. No water samples were collected for other chemical parameters.

Instantaneous discharge was determined at the survey reference locations. Total discharge was calculated by the incremental flow method using a standard top-setting rod and Marsh-McBirney Model 2000 velocity meter to obtain depth and water velocity data at numerous intervals across the stream.

Mussel Survey

ENVIRON staff conducted a mussel survey with assistance from Peabody Energy personnel in the WFBCMA on August 5, 2011. The survey incorporated two stream reaches, one positioned in the upper portion of the mitigation area and the other near the downstream portion of WFBC mitigation area (Figure 1). The survey followed standard techniques of Strayer and Smith (2003)

and used systematic sampling from random survey plots selected within a stratified distribution of transects. At each study reach, a reference location was selected, from which numerous cross-stream transects were established at uniform intervals in an upstream or downstream direction. At each cross-stream transect, a 0.25 M^2 sampling frame was positioned in the deepest portion of the stream flow (typically the center) to identify the sampling area (see Photo 5).

Mussel Survey Study Reaches

Figure 1 shows the location of the mussel survey study reaches in the WFBCMA. The reference location for the upper WFBCMA survey reach was latitude 39° 14.124' N and longitude -87° 21.616' W. Mussel sampling transects were positioned every 15 feet (ft) upstream of this location for a distance of 300 ft (see Photos 10 and 11). Due to mussel survey sampling results in the lower study area that was limited by the presence of a beaver dam, the upper mussel survey area was extended, and sampling transects were also positioned every 20 ft downstream of the reference location for a distance of 150 ft (see Photo 12). Thus, 29 sample plots for a total area of 7.25 M^2 were surveyed for mussels in the upper survey reach of the WFBCMA.

The reference location for the lower WFBCMA survey reach was latitude 39° 13.650' N and longitude -87° 21.434' W. This location is downstream of a beaver dam established since the 2010 bioassessment study and the location selected to best match flow conditions, stream width, and general gradient of the upper mussel survey area. Mussel sampling transects were positioned every 15 ft upstream of the reference location for a distance of 150 ft towards the beaver dam (Photo 16).

Mussel Survey Sampling

Following visual inspection within each 0.25 M^2 plot for surface mussels, substrate material was removed to a depth of 10-12 cm (4-5 inches) and placed onto a ¼ inch mesh screen for processing (Photos 5, 6, 7 and 8). This technique targeted greater than six-months to one-year old specimens to include large juvenile and adult stage mussels. The following information was recorded for each sample plot:

- Total width of stream at the transect location
- Number and species of mussels encountered
- Length (mm) of mussels encountered
- Substrate composition categories present (cobbles, mixed gravels, pea gravel, sand, mud, clay)

All live mussels were retained in a bucket of stream water until identified and measured, then returned to the stream unharmed. Specimens of dead and relic shells and valves were retained for identification verification and dead or relic shells that were observed along the stream bank were noted by distance from the reference point, measured, and identified when possible.

Mussel Survey Results

Water Quality

Water quality measurements in the upper survey reach for temperature, dissolved oxygen, and pH indicated typical ranges for a good quality mid-western stream demonstrating typical diurnal fluctuation. Water quality measurements taken during the mussel survey at the upper and lower survey reach reference locations are shown in Table 1. Water temperatures in the upper survey reach of West Fork Busseron Creek ranged from 28-34 °C with warmest temperature in mid-afternoon. Dissolved oxygen concentrations fluctuated with temperature saturation and in-stream algal productivity and ranged from 7.03-12.6 mg/L with highest concentrations in mid-afternoon. Specific conductivity and pH showed little temporal fluctuation and ranged from 260-296 mili-Semens/cm (µS/cm) and from 7.2-7.7 standard units (s.u.), respectively.

Water quality in the lower survey reach indicated both water temperature and dissolved oxygen concentration was influenced by the beaver dam (see Photos 13, 14, and 15). During the time of day when fluctuations of temperature and dissolved oxygen are typically near the diurnal peak, the water temperature (30.5 °C) and dissolved oxygen concentration (6.3 mg/L) were lower than observed at any time in the upper survey area (Table 1). The presence of the beaver dam did not appear to appreciably influence pH or specific conductivity. The slight increase in specific conductivity measured at the lower survey reach reference site is ecologically negligible, and may be a natural consequence of increased drainage area for this site.

Flow conditions at the time of the mussel survey reflected the dry meteorological conditions prevalent in the geographical area during several weeks prior to the survey. Flow at the upper study reach reference location was measured at 0.185 cubic feet per second (cfs), which increased to 0.323 cfs at the downstream survey reference location. The increase in flow downstream can be attributed to the contribution of local baseflow from the increased drainage area of the spoils. However, it is likely the beaver dam also serves as a reservoir that contributes to maintain flow in the lower portion of West Fork Busseron Creek.

Mussel Survey

Mussel survey results indicated two mussel species, *Utterbackia imbecillis* (Paper Pondmussel) and *Ligumia subrostrata* (Pondmussel) are present in the WFBCMA (see Photos 1-4). However, all mussel specimens recorded were encountered in the upper WFBCMA study reach and no mussel specimens were found in the lower WFBCMA study reach downstream of the beaver dam.

A total 25 sample plots were surveyed in the upper WFBCMA (upstream and downstream of the reference location), which accounted for 3% of the available stream habitat within the upper study reach. Eight live and 2 recently dead or relic *U. imbecillis* specimens were found within the sample plots compared to 15 live and one relic *L. subrostarata* individual. Remains of three *U. imbecillis* mussels were found outside the sample plots on the stream bank within the study area and their original location in the stream is unknown. Extrapolations for mussel density based on the mussels encountered within the sampling frame indicates approximate densities of 1.3 *U. imbecillis*/M^2 and 2.1 *L subrostrata*/M^2 within the study area. Table 2 provides a summary of the

mussel survey results and species density and length characteristics for the upper and lower WFBCMA stream reaches.

For the lower WFBCMA mussel survey reach a total of 10 survey plots were evaluated over a 150 ft length of stream (3% of stream area) with no mussels encountered. Based on the greater number of mussels encountered in the upper study reach for an equivalent 10-frame effort and length of stream, it was determined in the field to end the mussel survey in the downstream study area and extend the study area of the upstream survey reach. While no mussels were encountered in the study plots or observed along the shore at the downstream study reach, inference from the data indicate the density of mussels in this portion of the WFBCMA is much lower than observed in the upper study reach. Because mussels were encountered at the upper WFBCMA it cannot be stated with statistical confidence that mussels are completely absent within the lower survey area. The direct and indirect effects on the presence of mussels in the lower study reach from the beaver dam and recent deposition of bed materials are unknown.

A complete listing of the location, number, size, and predominant bed material for each sample plot is presented in Table 3 for both the upper and lower mussel survey study reaches of the WFBCMA. The mussel data showed no apparent relationship or patterns that could be detected between substrate characteristics and species of mussel encountered, or evidence of spatial trends in numbers or species of mussels in the upper survey area.

***Utterbackia imbecillis* Ecology**

This species is widespread in the Eastern half of the United States, with North to South ranges extending from Ontario Canada to South Texas. It is a very thin shelled, fast growing mussel species found in sandy to muddy bottomed slow creeks and ponds. Although this mussel species has been known to complete its life history without the use of fish hosts, the glochidia of this mussel are commonly found on sunfish, bullhead catfish, and large salamander larvae. This species is hermaphroditic, making it adaptable for early colonization of stream habitats. Specimens of this species in the 80 to 100mm range may be 3-5 years of age. Figure 2 shows the range in total valve length encountered for the specimens in WFBCMA.

***Ligumia subrostrata* Ecology**

This species has a similar distribution to the *Utterbackia,* however the common distribution is somewhat more centered within the Mississippi River drainage. It also prefers slow to still areas with sandy bottoms, but has slightly more of preference to cleaner sand and continuously flowing creeks than *Utterbackia*. The primary host species for this mussel are sunfish. The sexes of this species are identifiable by differences in shell morphology. This species is smaller at maturity than *Utterbackia*, potentially reaching maturity at two years of age and 30-40mm in length. Figure 2 shows the range in total valve length encountered for the specimens in WFBCMA.

Discussion

The finding of two species of live freshwater mussels at the numbers encountered and the size range of specimens verifies that mussels are established in the WFBCMA. Given the relatively complex life cycle and requirements for freshwater mussels to occur in streams (see above discussion of mussel ecology) the presence of *U. imbecillis* and *L. subrostrata* in the WFBCMA is further evidence that biological function of the stream has been attained. Furthermore, mitigation of this portion of West Fork Busseron Creek was completed in 2006 and the initial sighting of freshwater mussels in the WFBCMA was in 2010, suggesting a relatively fast rate of mussel recruitment and colonization. Successful colonization of two species of freshwater mussel over a 5-year period indicates that primary biological functions of the stream were likely present shortly following remediation.

Based on literature review of growth rates for similar unionid mussels we estimate the age of the *U. imbecillis* and *L. subrostrata* specimens encountered in the WFBCMA to be up to three years in age. Breeding season and glochidia release for many mussel species is in the spring through summer seasons, so it is estimated that the specimens retained by the ¼ inch mesh screen would have been produced no sooner than spring /summer of 2010.

Tables

Table 1. Water Quality Data West Fork Busseron Creek Mitigation Area, Farmersburg IN, August 5, 2011

Site	Time (hrs)	pH (su)	Temperature (°C)	Specific Conductivity (mS/cm)	Dissolved Oxygen (mg/L)	Flow (cfs)
Upper Survey Area [1]	1100	7.23	28.5	279	7.03	
Upper Survey Area [1]	1515	7.73	34.0	270	12.6	0.185
Lower Survey Area [2]	1715	7.52	30.5	296	6.32	0.323
Upper Survey Area [1]	1820	7.62	32.9	260	11.4	

1 Upper study reach reference location at lat: 32° 14.124' N and long: -87° 21.616' W
2 Lower study reach reference location at lat: 39° 13.650' N and long: -87° 21.434' W

Table 2. Mussel Survey Data West Fork Busseron Creek Mitigation Area, Farmersburg IN, August 5, 2011

Sample No.	Location From Site Reference (ft)	Live Mussel Taxa	Relect Mussel Taxa	Total Length (mm)	Substrate Characteristics
Upper WFBC Mitigation Area Mussel Survey Stream Reach					
21	300-upstream	*Utterbackia imbecillis*		15.6	mix gravel/sand
		Legumia subrostrata		22.5	mix gravel/sand
20	285-upstream	*Utterbackia imbecillis*		46.5	mix gravel/sand
on bank	283-upstream		*Utterbackia imbecillis*	80.2	recent dead
on bank	282-upstream		*Utterbackia imbecillis*	*85.3*	fragment, approx length
19	270-upstream	*Legumia subrostrata* ♂		44.5	cobble/gravel/sand
			Utterbackia imbecillis	*41.5*	partial, approx. length
18	255-upstream	none			mix gravel/sand
17	240-upstream	none			mix gravel/sand
16	225-upstream	none			mix gravel/sand
15	210-upstream		*Utterbackia imbecillis*	79.0	valve only, recent dead
		Legumia subrostrata		17.9	cobble/gravel/sand
14	195-upstream	none			cobble/gravel/sand
13	180-upstream	*Legumia subrostrata*		26.4	cobble/clay/sand
12	165-upstream	*Legumia subrostrata*		23.8	cobble/clay/sand
11	150-upstream	none			cobble/clay/sand
10	135-upstream	*Legumia subrostrata*		25.2	cobble/gravel/clay
			Legumia subrostrata		fragment, recent dead
9	120-upstream	*Utterbackia imbecillis*		63.4	pea gravel/clay
8	105-upstream	none			pea gravel/clay
7	90-upstream	*Legumia subrostrata*		26.5	pea gravel/clay
6	75-upstream	none			mix gravel/clay/sand
5	60-upstream	none			pea gravel/clay/silts
4	45-upstream	*Legumia subrostrata* ♀		24.8	mix gravel/clay/silts
3	30-upstream	none			mix gravel/clay/silts
2	15-upstream	*Legumia subrostrata*		18.7	mix gravel/sand
1	Reference [1]	none			mix gravel/sand
1d	15-downstream	none			mix gravel/sand
2d	35-downstream	none			sand/pea gravel
3d	55-downstream	*Utterbackia imbecillis*		17.8	sand/pea gravel
4d	75-downstream	none			mix gravel/sand
5d	95-downstream	*Utterbackia imbecillis*		20.7	mix gravel/sand
		Utterbackia imbecillis		17.6	mix gravel/sand
		Legumia subrostrata		33.7	mix gravel/sand
		Legumia subrostrata ♀		32.7	mix gravel/sand
		Legumia subrostrata		29.8	mix gravel/sand
on bank	103-downstream		*Utterbackia imbecillis*	*65*	partial, approx. length
6d	115-downstream	*Utterbackia imbecillis*		23.5	gravel/sand/silts
		Utterbackia imbecillis		20.7	gravel/sand/silts

Table 2. Mussel Survey Data West Fork Busseron Creek Mitigation Area, Farmersburg IN, August 5, 2011

Sample No.	Location From Site Reference (ft)	Live Mussel Taxa	Relect Mussel Taxa	Total Length (mm)	Substrate Characteristics
		Legumia subrostrata		31.9	gravel/sand/silts
		Legumia subrostrata		29.2	gravel/sand/silts
7d	135-downstream	none			sand/clay
8d	150-downstream	none			sand/clay
Lower WFBC Mitigation Area Mussel Survey Stream Reach					
	Reference [2]				
1	5-upstream	none			mix gravel/sand/clay
2	20-upstream	none			pea gravel/sand/clay
3	35-upstream	none			pea gravel/clay/sand
4	50-upstream	none			pea gravel/clay/sand
5	65-upstream	none			pea gravel/clay/sand
6	80-upstream	none			pea gravel/clay/sand
7	95-upstream	none			pea gravel/clay/sand
8	117-upstream	none			sand/mix gravel
9	130-upstream	none			sand/mix gravel
10	150-upstream	none			sand/mix gravel

1 Upper study reach reference location at lat: 32° 14.124' N and long: -87° 21.616' W

2 Lower study reach reference location at lat: 39° 13.650' N and long: -87° 21.434' W

Table 3. Summary of Mussel Survey Results, West Busseron Creek Mitigation Area, Farmersburg IN, August 5, 2011.

UPPER WFBC MITIGATION AREA	
Total wetted stream study area (M^2)	309
Total area surveyed for mussels (M^2)	7.25
Number of 0.25 M^2 survey plots	29 (2.3%)
Total number of mussels [1]	25
Total mussel species richness	2
Extrapolated mussel density (#/M^2)	3.4
Extrapolated *Utterbackia* density (#/M^2)	1.3
Mean *Utterbackia* valve length (mm)	27.3
Range *Utterbackia* valve length (mm)	15.6 - 85.3
Extrapolated *Legumia* density (#/M^2)	2.1
Mean *Legumia* valve length (mm)	35.2
Range *Legumia* valve length (mm)	18.7 - 33.7
LOWER WFBC MITIGATION AREA	
Total wetted stream study area (M^2)	82.4
Total area surveyed for mussels (M^2)	2.5 (3%)
Number of 0.25 M^2 survey plots	10
Total number of mussels [1]	0

1 Total number of live or dead mussels within sampling frame

Figures

Figure 1. West Fork Busseron Creek Mitigation Area (WFBCMA). Stream reach reference point locations for a mussel survey conducted on August 5, 2011. Photo shown is 2008 depiction of the mitigation area and current day stream channel, but does not reflect the final and current day restoration of the immediate watershed and buffer zone.

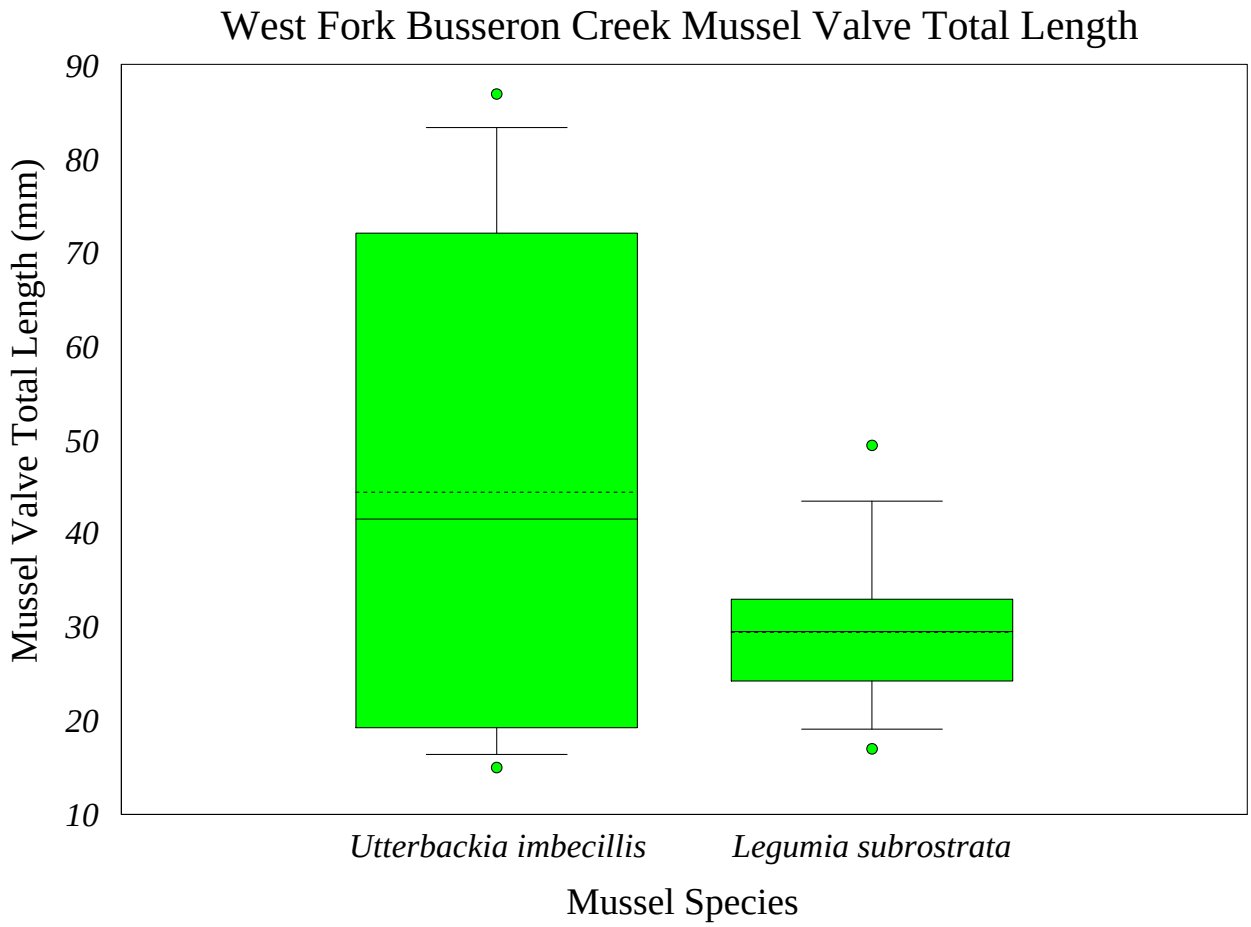

Figure 2. Box Plot of Mussel Valve Total Lengths. Values for mussels identified in survey plots in West Fork Busseron Creek, August 5, 2011. Upper and lower dots indicate 5th and 95th percentile range, the horizontal lines show the 90th and 10th percentile, the box shows the 25th to 75th percentile range, the dotted line indicates the mean valve length, and the solid line within the box indicates the median (50th percentile) valve length.

Photos

Photo 1. Fresh *Utterbackia imbecillis* from WFBC mitigation area on ¼ inch sieve screen portion of foot mantle extruded from valves.

Photo 2. *Utterbackia imbecillis* with valve length of 63.4 mm collected from WFBC mitigation area.

Photo 3. *Legumia subrostrata* (male) from WFBC mitigation area.

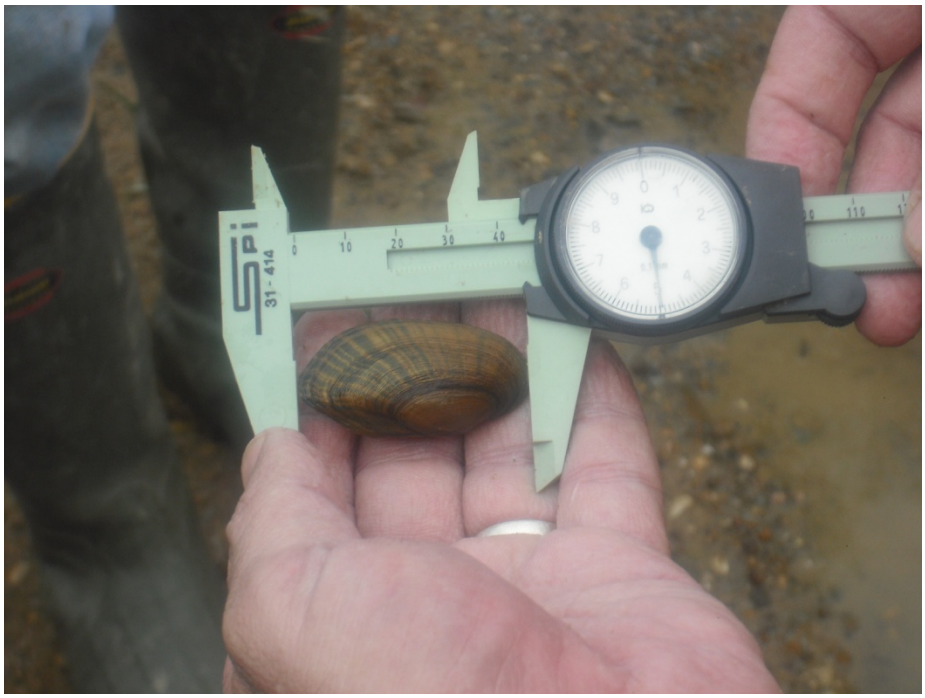

Photo 4. Ligumia subrostrata (male) with valve length of 44.5 mm from WFBC mitigation area.

Photo 5. Mussel survey 0.25 M^2 sampling frame and 0.25 in sieve screening pan.

Photo 6. Stream sediment from within mussel sampling frame being sieved in 0.25 in screening pan.

Photo 7. Field personnel checking screened substrate material for mussels.

Photo 8. *Utterbackia imbecillis* specimen from WFBC mitigation area in sieved mixed gravel on mussel screening pan.

Photo 9. Hinge view of *Utterbackia imbecillis* from WFBC mitigation area.

Photo 10. WFBC mitigation area upper mussel survey study area looking upstream from study reach reference point. August 5, 2011.

Photo 11. Typical stream conditions of WFBC mitigation area upper mussel survey study area. August 5, 2011.

Photo 12. Downstream terminus of upper WFBC mitigation area mussel survey study area, and immediately upstream of slack water due to grade control and beaver dam. August 5, 2011.

Photo 13. Downstream WFBC bioassessment mitigation area showing slack water due to presence of beaver dam. August 5, 2011.

Photo 14. WFBC mitigation area showing slack water due to presence of beaver dam. August 5, 2011.

Photo 15. WFBC mitigation area showing upstream limit of slack water due to presence of beaver dam. August 5, 2011.

Photo 16. WFBC mitigation area downstream mussel survey study area immediately downstream of beaver dam. August 5, 2011.